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BIOMATERIALS

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POSSIBILITIES OF USING COMPOSITE HYDROXYAPATITE CERAMICS AS CARRIERS OF CULTURED STEM CELLS

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The possibility of using a composite ceramic based on biogenic hydroxyapatite (BHA) as a carrier for cultured stem cells is examined. The results of investigations of the microstructure and biochemical activity of samples of BHA-based glass ceramic with the maximum open porosity, which were prepared for use as substrates for *in vitro* cloning fibroblast cells, are presented. The results of a visual assessment performed with a fluorescence microscope of cell adhesion and cell proliferation activity on bioresorptive membrane-type substrates made of a composite material based on biogenic hydroxyapatite are also presented.

Key words: biogenic hydrosilicates, hydroxyapatite ceramic, microstructure, biochemical activity, membrane-type substrates, carriers of cultivated stem cell colonies, cloned cells of fibroblasts, cell adhesion, proliferation activity.

The creation and development of carriers for cellular and bacterial cultures is a necessary step in the development of technology for medical materials science. The main factors having a positive effect on cell preservation and division are the bioactivity, texture, and porous structure of the carrier material.

It has been established that a de-mineralized bone matrix possesses stronger inductive properties than hydroxyapatite and tricalcium phosphate and is a better promoter of adhesion and proliferation of cultivated cell lines [1, 2]. The use of calcium phosphates combined with sodium or magnesium phosphates with the addition of silicon oxide has been studied in [3], and it has been established that these particular biomaterials have a positive effect on the differentiation of osteoblasts and osteogenesis.

Glass ceramic obtained on the basis of synthetic hydroxyapatite and medical sodium silicate glass can also be used as carriers for stem cells [4]. It has been established that the phase of active growth of a human diploid cell culture proceeds best with high open porosity and permeability of substrates consisting of the above-mentioned glass ceramics.

The study of the cloning effectiveness for precursor stem cells of human bone marrow on the biogenic hydroxyapatite (BHA) based glass-ceramic substrates obtained at the Institute of Materials Science of the Ukrainian National Academy of Sciences has shown that ceramics containing residual carbon have the best adaptation properties [5]. It has also been determined that such biomaterial implanted in children with damaged bone tissue accelerates osteogenesis as a result of enhanced bio-resorption and osteoconduction [6].

The high bioactivity of composite materials based on BHA and sodium-borosilicate glass is due to the particulars of the microstructure of the glass ceramic, which preserve the microstructure of the natural mineral in mammalian bones, identical to the microstructure of human bones. These minerals consist of nanograins assembled into micro- and macroagglomerates whose structure contains pores with an enormous size spectrum ranging from several tens of nanometers to hundreds of micrometers [7, 8]. The complex structure of individual crystalline BHA particles and the pores inhering in them remains in BHA-glass ceramic.

The aim of the present study was to prepare samples of BHA-based glass ceramic with maximum open porosity to check their use as membrane-type substrates, i.e., carriers to be used for *in vitro* cloning fibroblast cells.

EXPERIMENTAL OBJECTS AND PROCEDURE

The glass-ceramic samples used in the present work were prepared in the form of planar substrates with diameter about 15 mm by means of single-step sintering at temperatures

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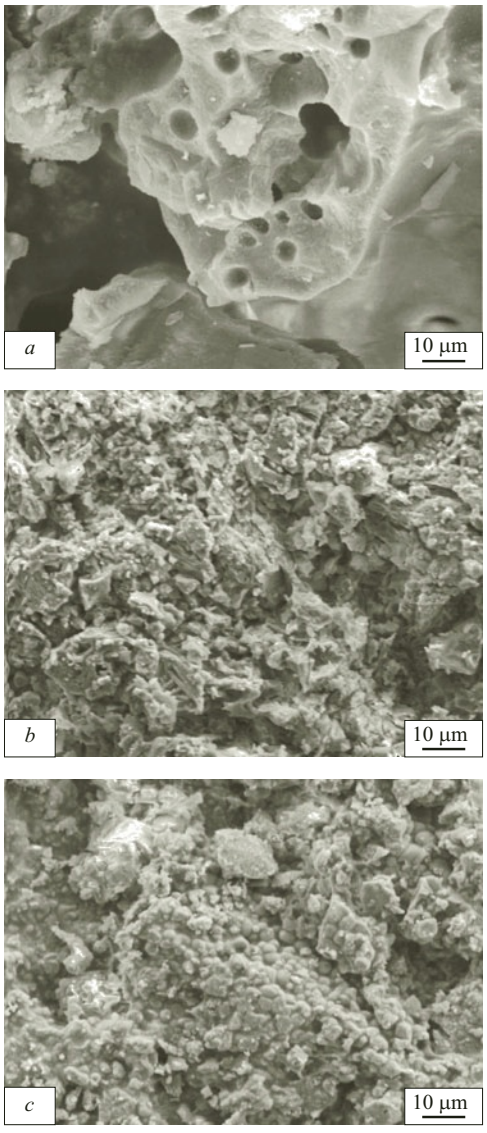

Fig. 1. Photomicrographs of glass-ceramic samples: *a*, *b*, *c*) samples Nos. 1, 2, and 3, respectively.

780 – 800°C of BHA-based composites with a definite content of sodium-borosilicate glass phase which are premolded from mixes with specially chosen granular composition of the components. The principal parameters are presented in Table 1, and the typical microstructures of the samples obtained are shown in Fig. 1.

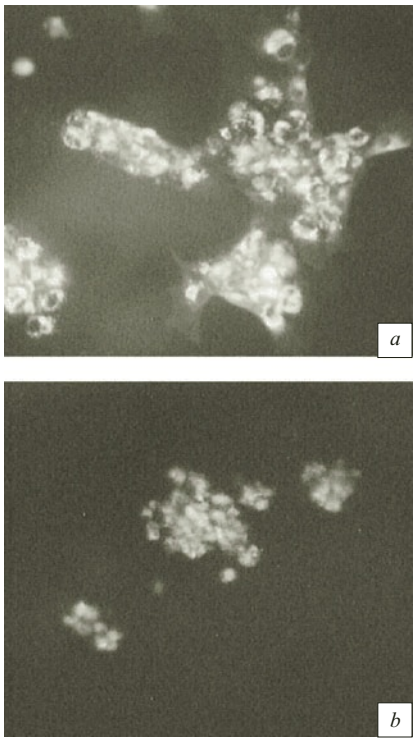

Fig. 2. Fibroblast culture on glass-ceramic sample No. 1. Fluorescence microscopy (× 200): *a*) first few days of culturing; *b*) five-day culture.

The glass ceramic samples were placed in Igla nutrient (Biolot, Russia) with pH = 8.45. The acidity of the medium was measured at the start and 2 and 24 h later. Subsequent measurements were performed after the medium was replaced (pH = 8.30). The fibroblast culture was stained with RKN67 Green intravital dye (Sigma, USA) and placed on carriers. The adhesion and proliferation activity of the cells were evaluated visually by means of fluorescence microscopy. After one week the old cells were removed with a 1 : 3 trypsin/Versene solution, the samples were placed into a new medium (pH = 8.20), the pH of the medium with the carriers was measured, and tagged fibroblasts were re-seeded on the samples.

RESULTS AND DISCUSSION

The glass-ceramic samples chosen for the present studies differed by the amount of glass phase and residual carbon. It

TABLE 1. Characteristics of Glass Ceramic Samples

Sample No.	Composition, wt.%			Apparent density, g/cm^3 (± 0.05)	Total porosity, % (± 0.05)	Open porosity, % (± 1.0)	Physiological solution pH	
	Glass phase	BHA	Carbon				after 5 min	after 2 h
1	70	30	≥ 0.15	1.22	55	36	6.2	8.8
2	To 32	68	–	1.47	49	40	10.4	11.2
3	To 32	68	≤ 0.07	1.42	50	45	8.8	9.4

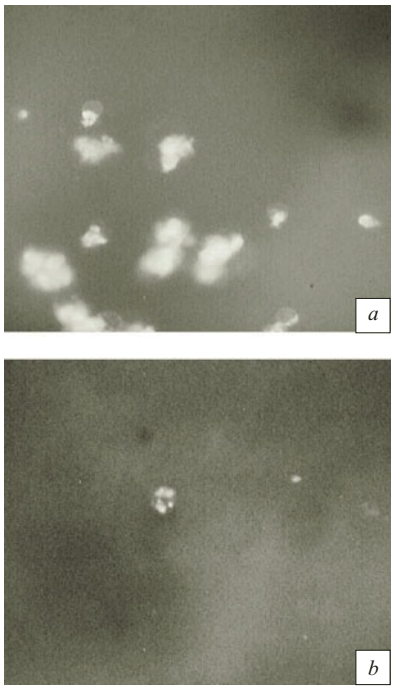

Fig. 3. Fibroblast culture on glass-ceramic sample No. 2. Fluorescence microscopy (× 200): *a*) first few days of culturing; *b*) five-day culture.

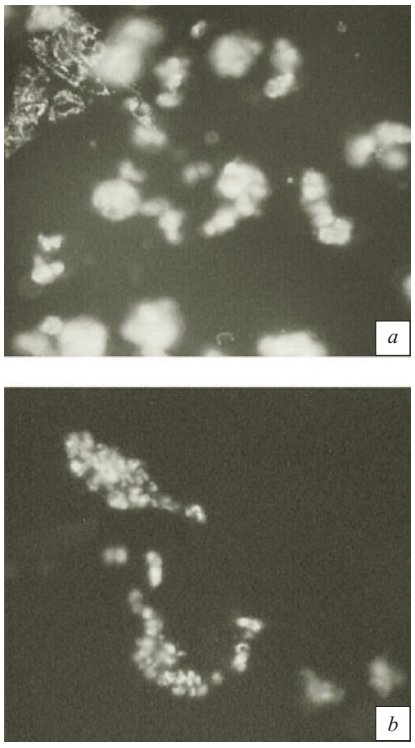

Fig. 4. Fibroblast culture on glass-ceramic sample No. 3. Fluorescence microscopy (× 200): *a*) first few days of culturing; *b*) five-day culture.

follows from Table 1 that the total porosity of each type of sample falls within the quite close limits which are required for biomaterials of this kind [4]. Analysis of the microstructure of the experimental samples shows that large pores with sizes > 10 μm and large crystalline BHA grains with transverse size ≥ 10 μm and even larger agglomerates of grains predominate in the samples No. 1 (Fig. 1*a*). The microstructure is qualitatively close with respect to the grain and pore sizes, which are predominately 5 – 15 μm (Figs. 1*b* and *c*). The open porosity is highest for samples No. 3. However, as will be shown below it is not the magnitude of this parameter that affects the result of cloning stem cells but rather the initial pH of the physiological solution or nutrient in which the samples are placed to perform experiments *in vitro* (Tables 1 and 2).

It was established that cell adhesion and proliferation on the surface of different samples occur differently. Fibroblasts adhere poorly on sample No. 2. Cell adhesion and proliferation occurred on samples Nos. 1 and 3, but on sample No. 3 the cells attached to the periphery of the carrier. The results of the studies are presented in Table 2 and Figs. 2 – 4.

The very weak attachment of the fibroblasts to the surface of samples No. 2 can be explained only by nutrient pH dominance, compared with other samples studied in the present work, during the first few days of culturing.

The number of fibroblasts remaining in the medium with samples Nos. 1 and 3 for the same observation times is approximately the same. As indicated above, these samples are close with respect to the grain and pore sizes and closer with respect to the initial pH of the media into which they were placed (see Tables 1 and 2). In all cases the sample No. 2 increases the initial pH of the media by the largest amount, and it is predominately this factor that negatively affects the cloning results.

TABLE 2. Change of the Nutrient pH with the Experimental Glass-Ceramic Samples

Sample No.	Change of the nutrient pH				
	at start of experiment with medium pH 8.45	after 2 h	after 24 h	after 25 h, in a new medium (pH = 8.30)	after 1 week, in new medium (pH = 8.20)
1	8.46	8.69	8.98	8.25	8.15
2	9.15	11.61	11.60	8.25	8.22
3	8.62	9.42	9.95	8.51	8.24

The positive effect of carbon, which is present in the samples Nos. 1 and 3 and decreases somewhat the pH of the media over the first days of their presence in the media into which they were placed compared with the effect of the samples No. 2, should be noted (see Tables 1 and 2).

CONCLUSIONS

It can be concluded on the basis of the results presented here that fibroblast adhesion and proliferation depend more on the biochemical activity than the porosity of the structure of the glass ceramic based on biogenic hydroxyapatite.

The presence of residual carbon in the glass ceramic composition of the type indicated also has a considerable effect on the bioactivity.

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